

b. enzymatically reducing said oxidation product to 2-KLG wherein said enzymatic reduction requires a reduced form of said enzymatic co-factor,

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between and coupled to the first oxidizing step and the reducing step wherein the oxidized form of said co-factor is selected from the group consisting of NADP⁺, NAD⁺, ATP, ADP, FAD and FMN,

wherein said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, and 6-carbon sugar acids, and is capable of being converted to an ascorbic acid (ASA) intermediate.

C1
cont
18.(Twice amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order:

a. enzymatically oxidizing the carbon source by a first oxidative enzymatic activity to a first oxidation product;

b. enzymatically oxidizing the first oxidation product by a second oxidative enzymatic activity to a second oxidation product;

c. enzymatically oxidizing the second oxidation product by a third oxidative enzymatic activity to a third oxidation product; and

C2
d. enzymatically reducing the third oxidation product by a reductase enzyme to 2-KLG

wherein at least one of said first, second and third oxidative enzymatic activities requires an oxidized form of an enzymatic co-factor and said reductase enzyme requires a reduced form of said enzymatic co-factor, wherein the oxidized form and the reduced form of said co-factor are recycled between and coupled to at least one oxidizing step and the reducing step

wherein the oxidized form of said co-factor is selected from the group consisting of NADP⁺, NAD⁺, ATP, ADP, FAD and FMN, and

wherein said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, 6-carbon sugar acids, and is capable of being converted to an ascorbic acid (ASA) intermediate .

C3
20.(Once amended) The process of Claim 18 wherein said first oxidative enzymatic activity requires an oxidized form of said enzymatic co-factor.

C4
25.(Once amended) ~~The process of Claim 18 wherein at least one of said first, said second, said third and said fourth enzymatic activities are immobilized.~~

C4
26.(Once amended) ~~The process of Claim 18 wherein at least one of said first, said second, said third and said fourth enzymatic activities are in solution.~~

C5
31.(Once amended) ~~The process of Claim 29 wherein said reductase activity is obtainable from *Corynebacterium* or *Erwinia*.~~

47.(Twice amended) ~~The process of Claim 15 or Claim 18 that is continuous.~~

48.(Twice amended) ~~The process of Claim 15 or Claim 18 that is batch.~~

49.(Twice amended) ~~The process of Claim 15 or Claim 18 that proceeds in an environment comprising organic solvents.~~

C6
50.(Twice amended) ~~The process of Claim 15 or Claim 18 that proceeds in an environment comprising long polymers.~~

51.(Twice amended) ~~The process of Claim 15 or Claim 18 further comprising the step of obtaining ASA from said 2-KLG.~~

63.(Once amended) A process for the non-fermentative production of 2-KLG from glucose comprising the following steps:

- a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;
- b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;
- c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and
- d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

C7
wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between and coupled to the glucose oxidizing step and the reducing step and wherein the oxidized form of said co-factor is NADP⁺ or NAD⁺.

73.(Once amended) A process for the non-fermentative production of 2-KLG from glucose comprising the following steps:

- a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;
- b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;
- c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and
- d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between and coupled to the glucose oxidizing step and the reducing step, and wherein the oxidized form of said co-factor is NADP⁺ or NAD⁺,

wherein the process proceeds in an environment wherein the 2,5-DKG reductase is provided exogenously to a host cell.

77.(Once amended) The process of Claim 80 wherein the host cells are viable.

78.(Once amended) The process of Claim 80 wherein the host cell is non-viable.

79.(Once amended) The process of Claim 80 wherein the host cell is modified to eliminate the naturally occurring GDH activity and a heterologous GDH having a specificity for NADP⁺ or NAD⁺ is introduced into said host cell.

Please add the following new claims.

80. A process for the non-fermentative production of 2-KLG in an environment comprising host cells, comprising the following steps in any order,

- a. enzymatically oxidizing glucose by a glucose dehydrogenase to produce a first oxidation product, wherein said oxidation requires an oxidized form of an enzymatic co-factor;
- b. enzymatically oxidizing said first oxidation product to produce a second oxidation product;
- c. enzymatically oxidizing said second oxidation product to produce a third oxidation product; and

DuV ~~d. enzymatically reducing said third oxidation product to 2-KLG, wherein said reduction requires a reduced form of said enzymatic co-factor~~

DuV ~~wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between and coupled to the first oxidizing step and the reducing step and said oxidized co-factor is NAD⁺ or NADP⁺ and said reduced co-factor is NADH or NADPH.~~

C 10 81. The process of Claim 80 wherein the oxidized co-factor is NAD⁺ and the reduced co-factor is NADH.

Cnt 82. The process of Claim 80 wherein the oxidized co-factor is NADP⁺ and the reduced co-factor is NADPH.

Add 24